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Analysis of the 'Spodoptera frugiperda' Nuclear Polyhedrosis Virus Genome by Restriction Endonucleases and Electron Microscopy

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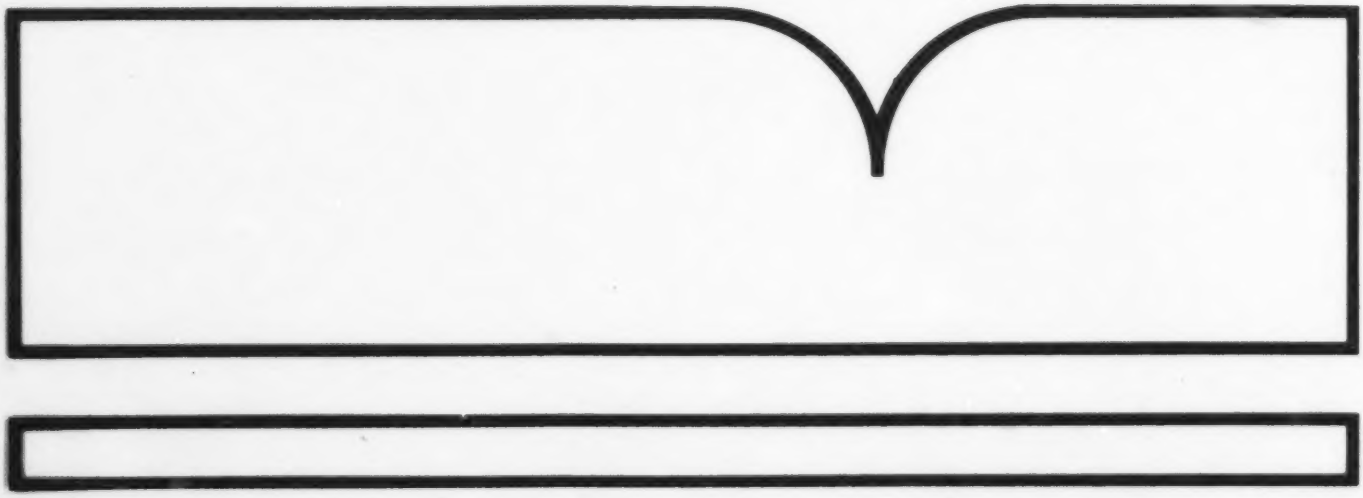

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16. ABSTRACT

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Analysis of the *Spodoptera frugiperda* Nuclear Polyhedrosis Virus Genome by Restriction Endonucleases and Electron Microscopy

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Restriction endonuclease analysis was used to differentiate between four strains of *Spodoptera frugiperda* nuclear polyhedrosis virus from different geographical areas. In addition, partial denaturation was performed, and a partial denaturation map was constructed for the Ohio strain of this virus.

With the increasing interest in the use of insect viruses as agents for the biological control of insect pests, there is an urgent need to identify and characterize insect viruses and various virus isolates. In this report, the restriction endonuclease patterns of DNA from a strain of *Spodoptera frugiperda* nuclear polyhedrosis virus (SfNPV) for *Bam*HI, *Eco*RI, and *Hin*dIII were determined and used to differentiate between SfNPV isolates from Georgia (GA), Mississippi (MS), North Carolina (NC), and Ohio (OH). In addition, a partial denaturation map of the OH strain of SfNPV was constructed.

The strains of SfNPV were originally isolated from diseased fall armyworm larvae at Tifton, Ga.; Starkville, Miss.; Plymouth, N.C.; and Cleveland, Ohio. The virions were purified from the lysate of virus-infected fall armyworm larvae by differential centrifugation and sucrose gradients as previously described (8). The extraction of DNA from the virions, its digestion by restriction endonucleases, and the in vitro labeling of DNA restriction fragments and their visualization after agarose gel electrophoresis were performed essentially as described in our previous report (8).

The partial denaturation map was constructed as follows. Purified viral DNA was partially denatured by a modification of the method of Inman and Schnos (3) as described by Wadsworth et al. (11) and Kilpatrick and Huang (5). Specifically, a 10-μl sample of DNA (6 to 10 μg/ml) was mixed with an equal volume of denaturation buffer at room temperature and allowed to react for 7 min. The denaturation buffer consisted of 20% (vol/vol) formaldehyde, 0.02 M Na_2CO_3, 5 mM EDTA, and enough NaOH to bring the pH up to an appropriate value. It was found empirically that a pH of 11.15 gave the most distinct partial denaturation pattern, and denaturation was already quite extensive at pH 11.25. The reaction was stopped by the addition of 80 μl of ice-cold spreading solution consisting of 70 μl of 1 M ammonium acetate, 5 μl of 0.2 M acetic acid, and 5 μl of cytochrome *c* (2 mg/ml) per 20 μl of the denatured DNA solution. The pH of the final solution was about 5.2.

The aqueous method (6) of spreading partially denatured DNA molecules (5, 11) was used to prepare the specimen grids. Immediately after the termination of partial denaturation, 1 μl each of denatured and completely alkaline-denatured ϕX174 RF molecules were added to the reaction mixture as internal length standards. A 50-μl amount of this solution was spread over the surface of an 0.3 M ammonium acetate solution adjusted to pH 5.2. The DNA-cytochrome *c* film was immediately transferred to parlodion-coated, 200-mesh copper grids by surface contact, stained with uranyl acetate, dehydrated in 90% ethanol, rotary shadowed with platinum-palladium (80:20) alloy, and stabilized with a carbon coating to minimize distortions from the electron beam.

The sample grids were examined in a Hitachi H-500 electron microscope at 50 kV. The electron micrographs of DNA molecules were taken at magnifications ranging from 3,000 to 9,000. The micrographs were enlarged by an overhead projector, and only intact, circular, relatively untangled DNA molecules were used for length measurements. A programmed Hewlett-Packard 9825A calculator and digitizer was used to trace the projected DNA molecules, and lengths were recorded in microns. ϕX174 RF DNA, with a

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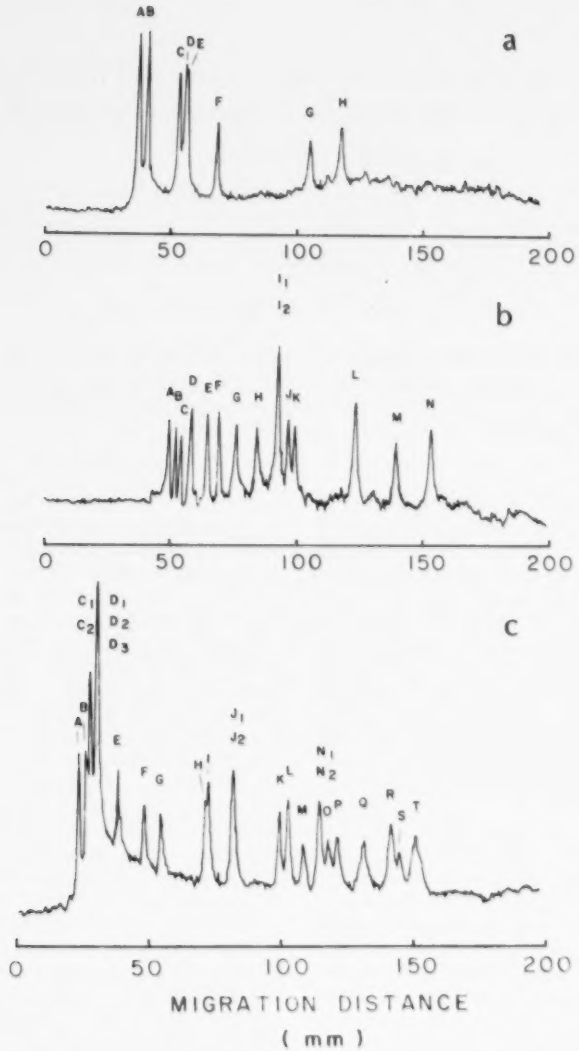

FIG. 1. Microdensitometer scans of autoradiographs of electrophoretically separated, end-labeled SfNPV OH DNA cleaved by (a) *Bam*HI, (b) *Hin*dIII, or (c) *Eco*RI. Fragment S in the *Eco*RI digest is the only fragment present in submolar (0.5 mol) amounts. All cleavage patterns were scanned at a 1:1 scan-to-record ratio.

known molecular weight of 3.48×10^6 (10), was used as a standard. For partially denatured molecules, the lengths of the single-stranded and double-stranded regions were measured separately. The lengths of the single-stranded regions were then corrected for shrinkage by a factor of 1.418, a value obtained empirically by comparing the molecular lengths of alkaline-denatured and intact φX174 RF DNA molecules cospread with the partially denatured SfNPV DNA molecules.

The OH strain of SfNPV was chosen for detailed analysis. The buoyant density of the viral DNA was found to be 1.6992 ± 0.0003 g/ml by equilibrium CsCl gradient centrifugation in a Spinco model E analytical ultracentrifuge, with *Micrococcus lysodeikticus* DNA used as a density marker (ρ = 1.731 g/ml). Thus, the viral DNA

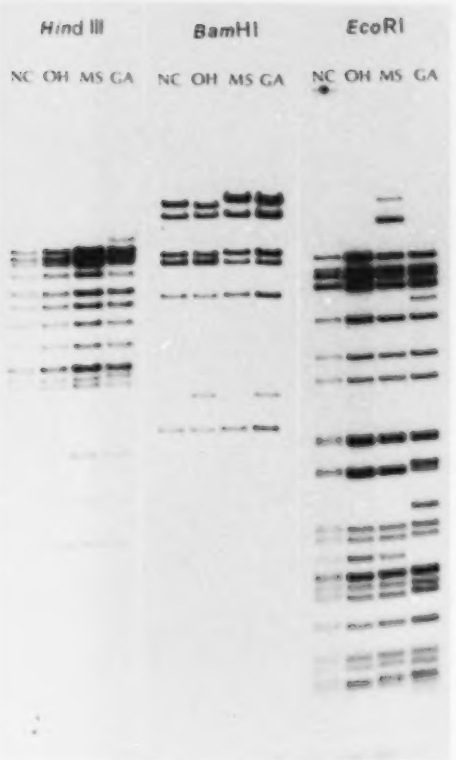

FIG. 2. Cleavage patterns of DNAs from the NC, OH, MS, and GA strains of SfNPV with restriction endonucleases *Bam*HI, *Hin*dIII, and *Eco*RI. The restriction fragments were end-labeled with [α-^{32}P]dATP and electrophoretically separated in an 0.7% agarose gel.

should have an average guanine plus cytosine (G+C) content of 40% as calculated by the equation derived by Schildkraut et al. (9). The molecular weight of the viral genome was found to be 82.5 ($\pm$5.2) $\times$ 10^6 by electron microscopy. By these parameters, it was virtually indistinguishable from the genome of the GA strain of SfNPV, from which the SfNPV strains propagated in most other laboratories in the United States and Europe were originally derived. The molecular weight and density data for SfNPV DNA obtained in our laboratory agree reasonably well with the values reported previously (1, 2, 4, 7).

The SfNPV OH genome was cleaved into 8, 15, and 25 fragments by the restriction endonucleases *Bam*HI, *Hin*dIII, and *Eco*RI, respectively (Fig. 1). The molecular weights of these restriction fragments and their designations were reported in a previous paper (8).

Viral DNA from the GA, MS, OH, and NC strains of SfNPV were cleaved with *Bam*HI, *Hin*dIII, or *Eco*RI, end-labeled, and electrophoretically separated on 0.7% agarose gels. The resulting autoradiographs are shown in Fig. 2. The migration patterns of the *Hin*dIII digests were identical for the MS, NC, and OH strains. The extra fragment present in the GA strain may be due to heterogeneity within the virus preparation. The *Eco*RI digests of the GA and MS strains had migration patterns that were easily distinguishable from those of the NC and OH strains. Heterogeneity may account for the presence of some of the submolar fragments observed. Loss of *Eco*RI sites, possibly between some of the linked comigrating fragments such as *Eco*RI fragments C and D, may also explain the appearance of extra high-molecular-weight restriction fragments (e.g., the *Eco*RI fragment above *Eco*RI-A in the MS digest [Fig. 1C and 2]).

Virions used for DNA purification were purified from the lysate of virus-infected larvae cloned in vivo. In vitro plaque purification of the various virus strains was not done because of the lack of a sound permissive cell system which can generate infectious virus in SfNPV-infected cell cultures. Therefore, confirmation of the loss of a specific restriction enzyme site must await DNA sequencing data and the construction of a complete restriction map of the viral genome for *Eco*RI. On the other hand, the migration patterns of the *Bam*HI digests were quite distinct for each of the four strains of SfNPV. The fact that SfNPV OH had the largest number of *Bam*HI sites was one reason this strain was chosen for detailed analysis and restriction mapping in our laboratory. From the known restriction map for *Bam*HI (8) and the sizes of the *Bam*HI restriction fragments, we can deduce that the NC strain might have lost the *Bam*HI site between *Bam*HI fragments A and G; the MS strain has lost *Bam*HI fragments A and D, and the GA strain has lost the *Bam*HI site between *Bam*HI fragments A and D (Fig. 1 and 2). Again, a final conclusion about the loss of *Bam*HI sites in these cases can only be made with the support of DNA sequencing data.

Preliminary experiments showed that the nick-translated *Bam*HI-G and D/E fragments eluted from gels of a *Bam*HI digest of SfNPV OH DNA did hybridize to the largest *Bam*HI restriction fragment of the other strains, as predicted.

To provide a means for orienting the circular viral DNA molecule, we constructed a partial denaturation map for the SfNPV OH genome. Partial denaturation of SfNPV DNA was initially

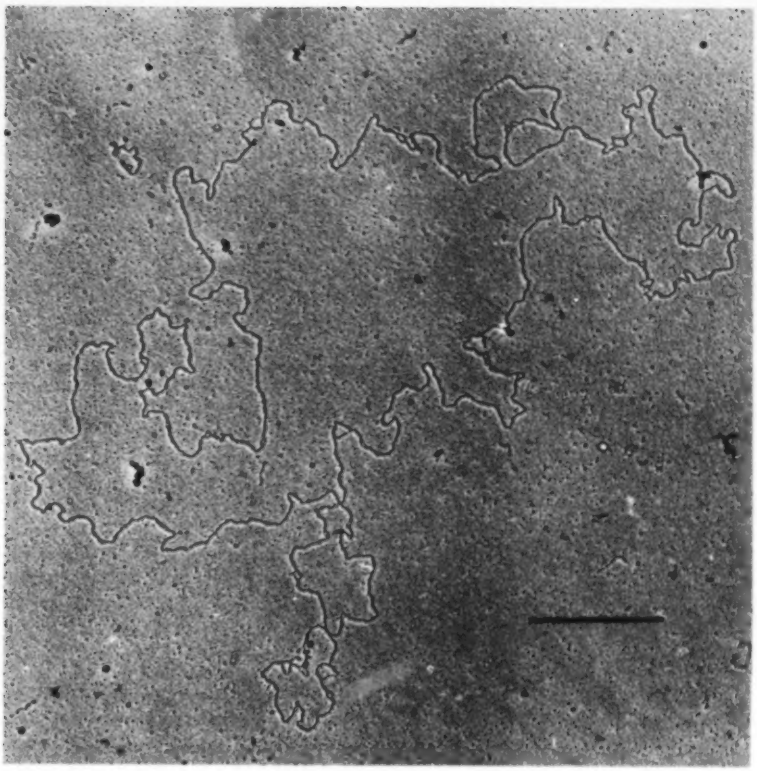

FIG. 3. Electron micrograph of a partially denatured SfNPV OH DNA molecule. Partial denaturation was accomplished in alkaline formaldehyde at pH 11.15 for 7 min at 25°C, and the DNA was spread by an aqueous method as described in the text. The small bubbles represent the denatured single-stranded regions. Bar, 1 μm.

performed at pH values ranging from 11.0 to 11.6, the reaction time being fixed at 7 min at room temperature (25°C). Small denatured regions were detectable as tiny "bubbles" along circular molecules at pH 11.15 (Fig. 3), but denaturation became extensive at pH 11.25. When the pH was raised above 11.25, extensive single-stranded regions were seen throughout the circular molecule, and distinct patterns were no longer discernible. Thus, it was not feasible to construct a precise partial denaturation map of the SfNPV genome because of the lack of a restriction enzyme which cleaves this DNA molecule at only one site. Therefore, the partial denaturation map of the SfNPV genome presented here was constructed from data obtained by denaturation at pH 11.15 and supplemented by data from denaturation at pH 11.25 by arbitrarily setting the major adenine plus thymine (A+T)-rich region of the molecule as the origin of the partial denaturation map during the alignment procedure described below.

We facilitated the data analysis by photographing only relaxed circular DNA molecules for length measurements. Because of the slight length variations between different DNA spreads, it was decided that the best way to compare the data from different experiments was to express all single- and double-stranded lengths as a percentage of total circular length instead of as absolute length units. It was found that with few exceptions, after correction for single-stranded shrinkage, the total circular lengths of partially denatured molecules were comparable to those of undenatured DNA molecules spread under similar conditions as controls. This justified the use of the shrinkage factor of 1.418 described above.

During the alignment of the partially denatured molecules, representations of these DNA molecules were plotted on strips of graph paper on a scale of 10% of the total length per inch. The molecules were then arranged for maximum overlap between the few A+T-rich and G+C-

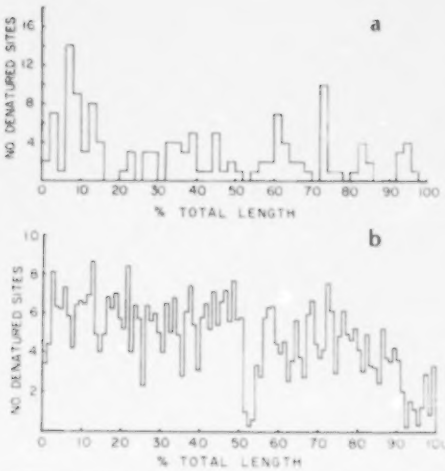

FIG. 4. Histograms showing the positions and frequencies of denatured sites for SfNPV OH DNA after partial denaturation at (a) pH 11.15 or (b) pH 11.25. The Y axis represents the number of denatured sites per 2% of the total length (a) or the number of denatured sites per 1% of the total length (b).

rich regions. The frequency of occurrence of the denaturation sites along the DNA of 14 molecules examined was then calculated to give the tentative partial denaturation map (Fig. 4).

Beginning from the origin of the map (Fig. 4a), there was a major relative A+T-rich zone which extended for about 15% of the total length. There followed a region of lower A+T content that stretched for about 30% of the molecule; at pH 11.25 (Fig. 4b) most of this region was denatured, but at pH 11.15 there were a few small, relatively G+C-rich sites scattered around this region. The first G+C-rich zone was found to be located immediately next to the central portion of the map and could only be recognized at pH 11.25. Another G+C-rich zone spanned the terminal 15% of the map. These two regions were the only ones that remained undenatured at pH 11.25. The region between them was marked by two relatively A+T-rich sites.

In summary, we characterized four geographically different strains of SfNPV by restriction endonuclease digestion. In addition, a partial denaturation map of the SfNPV OH genome was constructed. There was no indication of the presence of long stretches of high G+C or high A+T regions or of highly repetitive genome sequences, as was the case with certain herpesviruses. However, the asymmetrical pattern of the two G+C-rich regions shown in the denaturation map at pH 11.25 might provide a means for orienting the circular viral DNA molecule.

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